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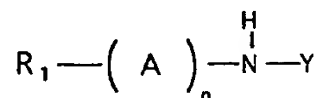
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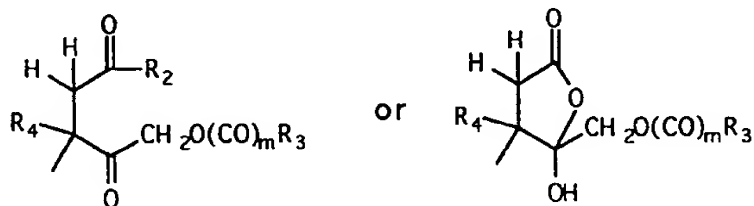
⑤ Peptide analogs as Irreversible Interleukin-1 β protease inhibitors.

⑤ Disclosed are compounds, compositions and methods for inhibiting interleukin-1 β protease activity, the compounds having the formula (I)



Formula I

wherein R_1 is an acyl group, as defined herein, A is an amino acid group as defined herein, n is 0-4 and Y is



This invention relates to peptide analogs that are interleukin-1 β protease inhibitors. More particularly, the invention provides α -substituted methyl ketones derived from aspartic acid and the closed hemi-ketal forms thereof as inhibitors of interleukin 1- β protease.

Enzymes involved in the catalytic degradation of proteins by hydrolyzing peptide bonds are known as proteases or proteinases. Proteinases are believed to be involved in various disease states including inflammation, metastasis, tissue damage, bone resorption and muscle degeneration in dystrophic diseases. Proteinases are divided into classes according to their catalytic mechanisms, such as serine-, cysteine-, aspartic- and metallo-proteinases. For each class of proteinases, the catalytic site of the enzyme lies in the cleft on the surface of the enzymes in which reside the specificity subsites that bind amino acid side chains and the polypeptide backbone. In designing proteinase inhibitors, it is important to optimize the subsite binding characteristics with appropriate amino acid substrate analogs.

This invention relates to peptide substrates modified with affinity labels that inhibit interleukin-1 β protease (hereinafter IL-1 β protease). These inhibitors are thought to act by alkylating the cysteine sulfhydryl group (cys 285) within the catalytic site of IL-1 β protease. Affinity labeling has been used since the 1960's to prepare irreversible peptide-based inhibitors which act to alkylate the active sites of cysteine proteases. A variety of affinity labels and amino acid sequences have been synthesized to improve the binding of these modified peptide inhibitors to the enzyme's active site. These affinity labels include peptidyl halomethyl ketones, peptidyl diazomethyl ketones, epoxysuccinyl peptides and peptidyl methylsulphonium salts as reviewed by D. Rich in Chapter 4 of "Proteinase Inhibitors", Barret, A.J. and Salvesen, G., eds., Elsevier, 1986. More recently, peptide acyloxymethyl and aryloxymethyl ketones have also been described as affinity labels (Krantz, A. *et al.*, Biochemistry, 30, p. 4678-4687, 1991). Current research (see for example European Patent Application, Pub. No. 015,748 A2; PCT International Publication No. WO 91/15577; Chapman, K.T., Biorganic & Medicinal Chem. Lett. 1992, 2, 613-618) has been directed towards understanding the enzyme binding specificity requirements in designing novel small molecular weight protease inhibitors that are efficacious, safe and have specificity for IL-1 β protease which is believed to play an important role in many disease states (see Epstein, F.H., New Engl. Jrl. of Med., 328, p. 106-113, 1993).

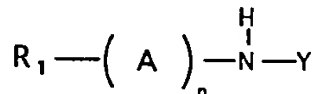
Disease states in which IL-1 β protease inhibitors may be useful as therapeutic agents include: infectious diseases, such as meningitis and salpingitis; septic shock, respiratory diseases; inflammatory conditions, such as arthritis, cholangitis, colitis, encephalitis, endocervicitis, hepatitis, pancreatitis and reperfusion injury, immune-based diseases, such as hypersensitivity; auto-immune diseases, such as multiple sclerosis; bone diseases; and certain tumors.

It is an object of the present invention to provide novel peptidyl substrate analogs modified with electronegative leaving groups that bind at the active site of IL-1 β protease and inhibit IL-1 β protease activity. IL-1 β protease cleaves a biologically inactive 34kD precursor of IL-1 β to form the biologically active 17kD cytokine. This cleavage occurs at the peptidyl sequence of Val-His-Asp/Ala-Pro-Val.

It is another object of the present invention to provide compositions comprising the above-referred to compounds.

It is a further object of the present invention to provide a method of use of the composition for the treatment of the above-identified disease states.

According to the present invention, there is provided a compound of the formula (I) and a pharmaceutically acceptable salt thereof:

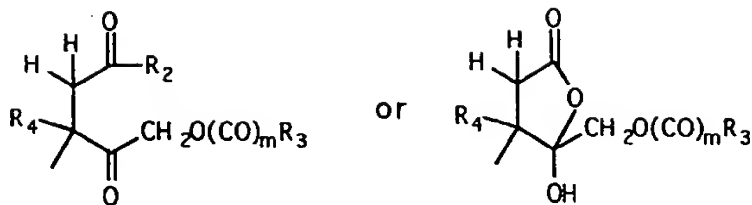


Formula I

wherein

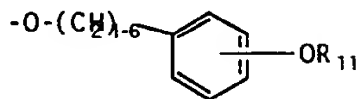
$n = 0-4$;

$Y =$



10
m =
R₃ =

0,1;
a singularly or multiply substituted aryl wherein aryl is a phenyl or naphthyl ring wherein the substituents are independently selected from the group consisting of:
(1) H; (2) halogen; (3) OH
(4) CF₃; (5) NO₂; (6) OR₅
(7) COR₈; (8) NR₆ COR₁₀; (9) CONR₅ R₆
(10) SO₂NR₅ R₆; (11) SO₂R₆; (12) COOR₁₁;
(13)



25
and
(14) lower alkyl and lower cycloalkyl;

30
R₅ =

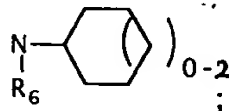
(1) lower straight chain or branched alkyl, lower cycloalkyl
(2) (CR₆ R₇)₀₋₆-aryl; (3) (CR₆ R₇)₀₋₆-heteroaryl or
(4) (CR₆ R₇)₂₋₆-R₈;

R₆ and R₇

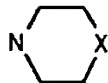
are independently H, lower straight chain or branched alkyl, benzyl, cycloalkyl or aryl wherein aryl is defined as above and heteroaryl includes pyridyl, thienyl, furyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, benzimidazolyl, pyrazinyl, pyrimidyl, quinolyl, isoquinolyl, isothiazolyl, benzofuranyl, isoxazolyl, triazinyl and tetrazolyl;

35
R₈ =

(1) OCH₂CH₂OR₆; (2) OCH₂CH₂NR₆ R₇
(3) NR₆CH₂CO₂R₆;
(4)



45
(5)



X = O,S and

(6) NR₆ R₇ wherein R₆ and R₇ are as above defined;

55
R₉ =

(1) lower straight chain or branched alkyl, lower cycloalkyl
(2) (CR₆ R₇)₀₋₆-aryl; (3) (CR₆ R₇)₀₋₆-heteroaryl; or

$R_{10} =$ (4) $(CR_6R_7)_{0-6}-R_8$, wherein R_6 , R_7 and R_8 are as above defined;

(1) R_9 ; (2) OR_{11} ; or (3) NR_6R_{11} ,

wherein

5 $R_{11} =$

(1) lower straight chain or branched alkyl, lower cycloalkyl

(2) $(CR_6R_7)_{1-6}$ -aryl; (3) $(CR_6R_7)_{1-6}$ -heteroaryl; or

(4) $(CR_6R_7)_{2-6}-R_8$, and R_6 , R_7 , R_8 and R_9 are as above defined;

10 $R_4 =$

H or deuterium;

$R_2 =$

(1) OR_8 ; (2) NR_6OR_7 or

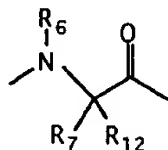
(3) NR_6R_7 , and R_6 and R_7 are as above-defined;

A =

15

(1) an amino acid of the formula (II)

20



Formula (II)

25

wherein R_6 and R_7 are as defined above;

R_{12}

is independently

(1) H or; (2) $(CR_6R_7)_{1-6}-R_{13}$, and R_6 and R_7 are as above-defined;

30

$R_{13} =$

(1) H; (2) F; (3) CF_3

(4) OH; (5) OR_{11} ; (6) NR_6R_{14}

(7) cycloalkyl; (8) aryl

(9) heteroaryl; (10) SH

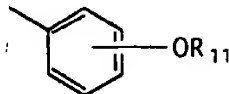
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(11) SR_{11} ; (12) $CONR_5R_6$

(13) $COOR_5$ or

(14)

40



45

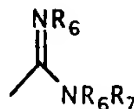
wherein R_5 , R_6 , R_{11} and R_{14} are as defined above;

$R_{14} =$

(1) R_7 ; (2) COR_{10} ; (3) $SO_2NR_5R_6$ or

(4)

50

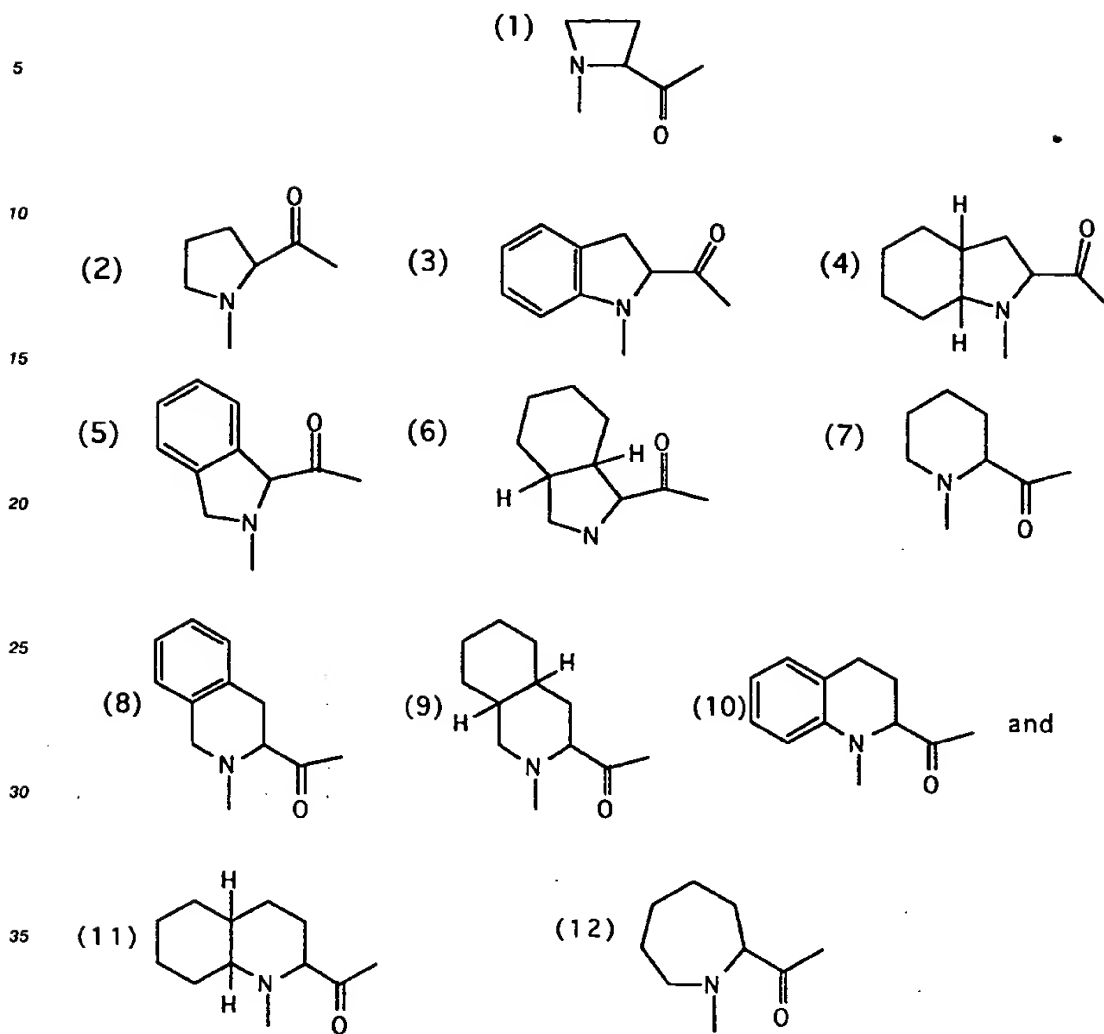


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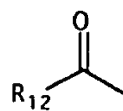
wherein R_5 , R_6 , R_7 and R_{10} are as defined above; or

A =

(2) an amino acid selected from the group consisting of



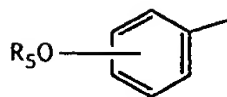
R_1 is an acyl group of the formula (III)



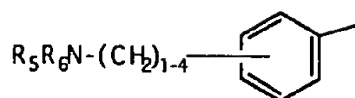
Formula III

wherein
 R_{12} is

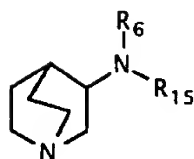
- (1) OR_5 ; (2) NR_5R_6 ; (3) R_5 ; (4) $-CH=CHR_5$
 (5)



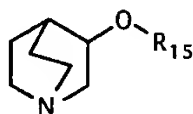
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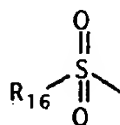
(7)



or
(8)

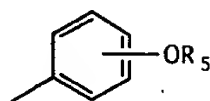


wherein
 R_{15} = single bond, $(CH_2)_{2-6}-NR_6-$, $(CH_2)_{2-6}-O-$ and R_5 and R_6 are as above defined; or a sulfonyl group of the formula (IV)

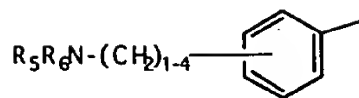


Formula IV

wherein
 R_{16} is
 (1) R_5
 (2)



or
(3)



5

wherein R_5 and R_6 are as above-defined.

As used herein the term pharmaceutically acceptable salts include the acid and base addition salts.

The term acid addition salts refers to those salts which retain the biological effectiveness and properties
 10 of the free bases and which are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

15 The term base addition salts include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium and magnesium salts derived from pharmaceutically acceptable organic non-toxic bases including salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion
 20 exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, trimethamine, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaines, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. Particularly preferred organic non-toxic bases are isopropylamine, diethylamine, ethanolamine,
 25 trimethamine, dicyclohexylamine, choline and caffeine.

Alkyl means a saturated or unsaturated aliphatic hydrocarbon which may be either straight- or branched-chain. Preferred groups have no more than about 12 carbon atoms and may be methyl, ethyl and structural isomers of propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl and dodecyl.

Lower alkyl means an alkyl group as above, having 1 to 7 carbon atoms. Suitable lower alkyl groups are
 30 methyl, ethyl, n-propyl, isopropyl, butyl, tert-butyl, n-pentyl, neopentyl, n-hexyl, and n-heptyl.

Substituted phenyl means a phenyl group in which one or more of the hydrogens has been replaced by the the same or different substituents including halo, lower alkyl, nitro, amino, acylamino, hydroxyl, lower alkoxy, aryl, heteroaryl, lower alkoxy, alkylsulfonyl, trifluoromethyl, morpholinoethoxy, morpholinosulfonyl, and carbobenzoxy-methyl sulfamoyl.

35 Halogen means chloride, fluoride, bromide or iodide.

Lower cycloalkyl means cycloalkyl having C_3 to C_6 carbon atoms.

* In another aspect of the invention there is provided a method of inhibiting interleukin-1 β protease activity in a mammal in need of such treatment comprising the administration to the mammal of an effective inhibitory amount of a pharmaceutical composition of a compound of formula (I) or a pharmaceutically
 40 acceptable salt thereof as the active agent. IL-1 β mediated disease states and disorders which can be thus treated include: infectious diseases, such as meningitis and salpingitis; septic shock, respiratory diseases; inflammatory conditions, such as arthritis, cholangitis, colitis, encephalitis, endocervicitis, hepatitis, pancreatitis and reperfusion injury, immune-based diseases, such as hypersensitivity; auto-immune diseases, such as multiple sclerosis; bone diseases; and certain tumors.

45 In the practice of this invention an effective amount of a compound of the invention or a pharmaceutical composition thereof is administered to the subject in need of, or desiring, such treatment. These compounds or compositions may be administered by any of a variety of routes depending upon the specific end use, including orally, parenterally (including subcutaneous, intraarticular, intramuscular and intravenous administration), rectally, buccally (including sublingually), transdermally or intranasally. The most suitable
 50 route in any given case will depend upon the use, the particular active ingredient, and the subject involved. The compound or composition may also be administered by means of controlled-release, depot implant or injectable formulations as described more fully herein.

55 In general, for the uses as described in the instant invention, it is expedient to administer the active ingredient in amounts between 0.1 and 100 mg/kg body weight, most preferably from 0.1 to 30 mg/kg body weight for human therapy, the active ingredient being administered preferably in the range of from 0.1 to 20-50 mg/kg/day. This administration may be accomplished by a single administration, by distribution over several applications or by slow release in order to achieve the most effective results. When administered as a single dose, administration will most preferably be in the range of from 0.1 to 10 mg/kg of body weight.

The exact dose and regimen for administration of these compounds and compositions will necessarily be dependent upon the needs of the individual subject being treated, the type of treatment, and the degree of affliction or need. In general, parenteral administration requires lower dosage than other methods of administration which are more dependent upon absorption.

5 A further aspect of the present invention relates to pharmaceutical compositions comprising as an active ingredient a compound of the present invention of formula (1) in admixture with a pharmaceutically acceptable, non-toxic carrier. As mentioned above, such compositions may be prepared for use for parenteral (subcutaneous, intraarticular, intramuscular or intravenous) administration, particularly in the form of liquid solutions or suspensions; for oral, rectal or buccal administration, particularly in the form of tablets
10 or capsules; transdermally; or intranasally, particularly in the form of powders, nasal drops or aerosols.

When administered orally (or rectally) the compounds will usually be formulated into a unit dosage form such as a tablet, capsule, suppository or cachet. Such formulations typically include a solid, semi-solid or liquid carrier or diluent. Exemplary diluents and vehicles are lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, mineral oil, cocoa butter, oil of theobroma, alginates, tragacanth,
15 gelatin, syrup, methylcellulose, polyoxyethylene sorbitar monolaurate, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, and magnesium stearate.

The compositions may be prepared by any of the methods well-known in the pharmaceutical art, for example as described in Remington's Pharmaceutical Sciences, 17th edition, Mack Publishing Company, Easton, PA, 1985. Formulations for parenteral administration may contain as common excipients sterile
20 water or saline, alkylene glycols such as propylene glycol, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Examples of vehicles for parenteral administration include water, aqueous vehicles such as saline, Ringer's solution, dextrose solution, and Hank's solution and nonaqueous vehicles such as fixed oils (such as corn, cottonseed, peanut, and sesame), ethyl oleate, and isopropyl myristate. Sterile saline is a preferred vehicle and the compounds are
25 sufficiently water soluble to be made up as a solution for all foreseeable needs. The vehicle may contain minor amounts of additives such as substances that enhance solubility, isotonicity, and chemical stability, for example, antioxidants, buffers, and preservatives. For oral administration, the formula can be enhanced by the addition of bile salts and also by the addition of acylcarnitines (Am. J. Physiol. 251:332 (1986)). Formulations for nasal administration may be solid and contain as excipients, for example, lactose or dextran, or may be aqueous or oily solutions for administration in the form of nasal drops or metered spray.
30 For buccal administration typical excipients include sugars, calcium stearate, magnesium stearate, pregelatinated starch, and the like.

When formulated for nasal administration the absorption across the nasal mucous membrane is enhanced by surfactant acids, such as for example, glycocholic acid, cholic acid, taurocholic acid,
35 desoxycholic acid, chenodesoxycholic acid, dehydrocholic acid, glycodeoxycholic acid, and the like. (See, B.H. Vickery, "LHRH and its Analogs-Contraception and Therapeutic Applications", Pt. 2, B.H. Vickery and J.S. Nester, Eds., MTP Press, Lancaster, UK, 1987).

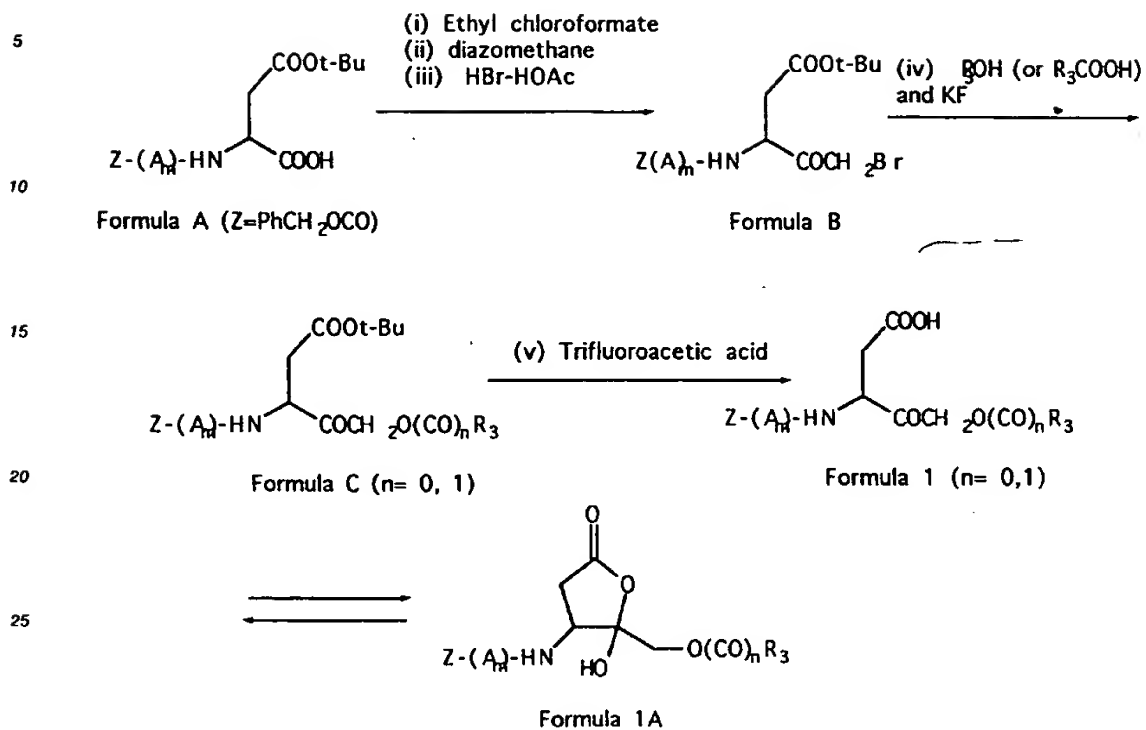
Compounds of the present invention are prepared using the procedure described generally in Schemes I, II and III and in more detail as described in the Examples, but the scope of the invention is in no way to
40 be construed as limited thereto.

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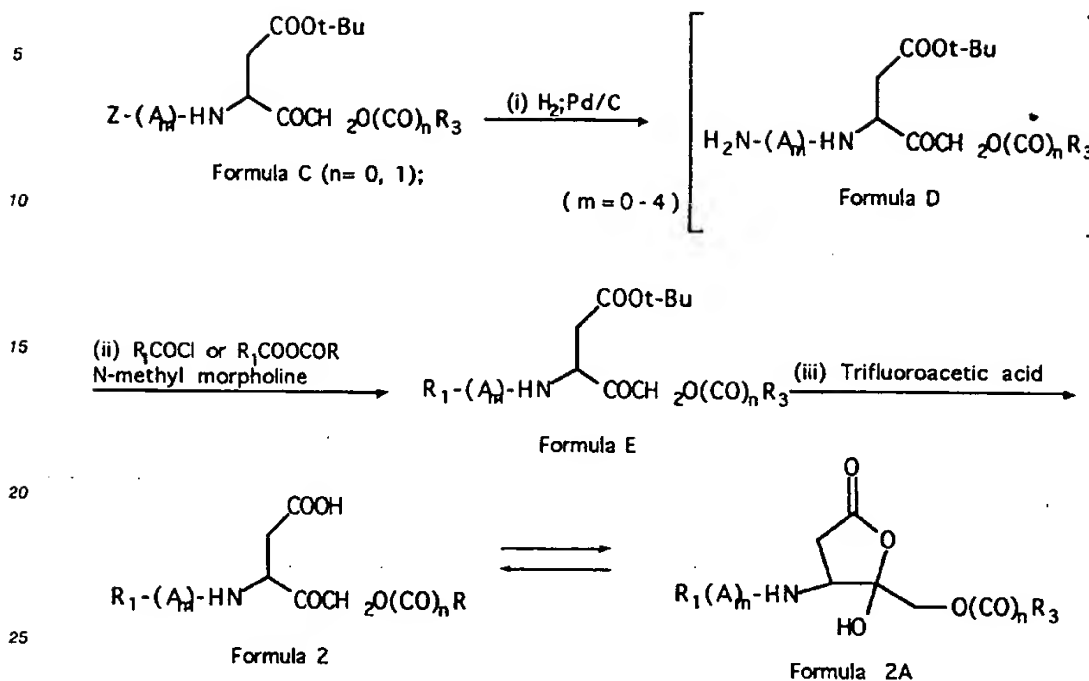
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Scheme 1



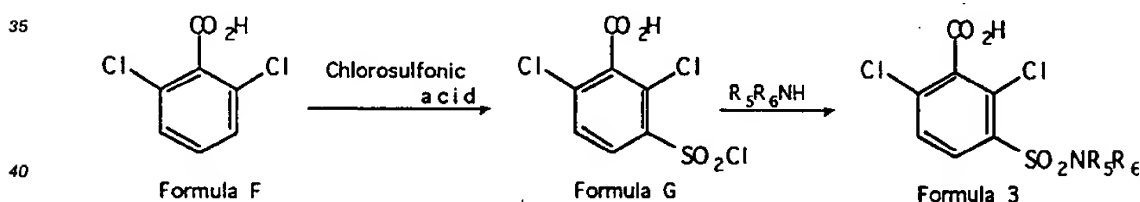
wherein A, R_3 and m are as defined in formula (I) and Z is benzyloxycarbonyl.

Scheme II



wherein Z, A, R_1 , R_3 , m and n are as defined in formula (I).

Scheme III



The synthesis of the disclosed interleukin enzyme (ICE) inhibitors was conducted by one of two methods depicted in Schemes I and II. For inhibitors which contained an N-terminal benzyloxycarbonyl group ("Z" group), N-benzyloxycarbonyl-L-aspartic acid mono t-butyl ester or other benzyloxycarbonyl protected aspartic acid-based peptides (Formula A) were used as starting materials. The synthesis of the requisite peptides are readily carried out by a variety of methods known to those practicing in the art of peptide chemistry. The aspartic acid-based peptide (Formula A) is reacted with ethyl chloroformate and N-methyl morpholine in tetrahydrofuran (THF) at low temperature (ca. $-15^{\circ}C$) for approximately 30 min. This generates a mixed anhydride in solution thereby activating the free carboxylate toward nucleophilic attack. Other activating reagents (for example isopropyl chloroformate), solvents (diethyl ether, dioxane), and tertiary amine bases (diisopropyl ethylamine, triethylamine) can be used in place of the above preferred reagents to form a reactive carboxylate species. The pre-formed mixed anhydride is treated (without isolation) with a solution of diazomethane in diethyl ether. The diazomethane reagent is prepared under standard conditions from DIAZALD® using a commercially available (Aldrich) diazomethane generator. A one to two molar excess of diazomethane is added and the reaction mixture is warmed from $-15^{\circ}C$ to $25^{\circ}C$ over a 20 min period. During this time, diazomethane reacts with the mixed anhydride to form an α -

diazoketone. The α -diazoketone is not isolated and the reaction mixture is treated directly with an excess of a 1:1 solution of 48% hydrobromic (HBr) and glacial acetic (HOAc) acids. The mixture of acids are added dropwise to the α -diazoketone and the reaction mixture is subsequently stirred for at least 15 minutes. This treatment with 1:1 48% HBr and glacial HOAc decomposes the α -diazoketone to yield the desired N-benzyloxycarbonyl-L-aspartic acid mono t-butyl ester α -bromoketone (Formula B) and nitrogen gas as a by-product. The bromomethyl ketone is typically isolated as an oil using standard procedures which are apparent to those skilled in the art. The α -bromoketone so obtained is of sufficient purity to be used in all subsequent reactions. However, the ketone can be further purified by high pressure liquid chromatography (HPLC), if analytically pure material is desired.

The t-butyl ester α -bromoketone (Formula B) is subsequently reacted with a variety of phenols, naphthols, and arylcarboxylic acids. This is conducted by exposing the bromomethyl ketone to an excess of the phenol, naphthol or arylcarboxylic acid in dimethylformamide containing sodium or potassium hydride or potassium fluoride. The reaction can be conveniently monitored by thin layer chromatography (TLC) and once the TLC indicates that displacement of the bromide with the phenol or carboxylate is completed, the product is isolated using standard procedures. The desired aspartic acid mono t-butyl ester α -aryloxymethyl- or α -arylacetyloxymethyl ketone (Formula C) may be purified by conventional methods including recrystallization and silica gel column chromatography.

The remaining synthetic transformation to generate the ICE inhibitors is the hydrolysis of the t-butyl ester function. This is conducted by exposing the t-butyl ester (Formula C) to a 25% solution of trifluoroacetic acid (TFA) in methylene chloride at 25 °C. The de-esterification is typically complete within 3 hrs. Removal of the volatile TFA and organic solvent affords the aspartic acid (Formula 1). The yield of the reaction is quantitative in most instances, providing the t-butyl ester starting material is of high purity. Purification, if required, can be performed by recrystallization or chromatographic techniques which are well known to those skilled in the art. The concentration of TFA may range from 5%-100% and other organic solvents may be used such as chloroform. Alternatively, a solution of 3M anhydrous hydrochloric acid in ethyl acetate may be used in place of the TFA-methylene chloride solution with equal efficiency. The ¹H NMR spectra of these acids of Formula 1 indicate that they exist in equilibrium as the closed hemiketal form shown in Formula 1A and that the ratio of Formula 1 versus Formula 1A is solvent-dependent.

In Scheme II, the synthesis of aryloxy- and arylacetyloxymethyl ketones (Formula 2) which possess an N-terminal group other than the Z-group are described. The aspartic acid derivatives of Formula C are the starting material for the synthesis of inhibitors of Formula 2. First the Z-group is removed to generate the N-terminal amine (Formula D) under hydrogenolytic conditions. The reagents and conditions typically used to carry out the hydrogenolytic removal of the Z-group are hydrogen gas, ambient temperature conditions and pressure, 5% palladium on carbon as the catalyst in an alcoholic solvent for example, methanol, optionally containing two equivalents of hydrochloric acid. It is not necessary to purify the intermediate free amine (or the hydrochloride salt if hydrochloric acid is used in the hydrogenolysis), though this material needs to be dry and free of alcohol for the subsequent coupling reaction to proceed in good yield.

The N-terminal amine is then condensed with a carboxylic acid to yield intermediates of Formula E. It is generally necessary to first activate the acid as an acid chloride or mixed anhydride and then react it with the free amine (or hydrochloride salt) in the presence of an organic base, for example, N-methylmorpholine. Alternatively, coupling with acid with the intermediate amine is conducted using amide coupling reagents/conditions employed in peptide coupling chemistry ("The Practice of Peptide Synthesis", M. Bodanszky, Springer-Verlag, NY, 1984; "The Peptides", Vol 1-3, E. Gross and J. Meienhofer, Eds. Academic Press, NY, 1981). Lastly, the t-butyl ester in Formula E is removed with trifluoroacetic acid (as described above) to give the aspartic acid analogs of Formula 2. As in the case of the compounds of Formula 1, the ¹H NMR of components of Formula 2 appear to exist in equilibrium with their corresponding closed hemiketal counterparts of Formula 2A.

The phenols, naphthyls and arylcarboxylic acids used in the reaction with the bromomethyl ketones can be either purchased from commercial sources or synthesized by adopting known procedures. Their synthesis would be readily deduced by those skilled in the art of organic synthesis. By way of example, the preparation of the 2,6-dichloro-3-sulfonamidobenzoic acids are presented in Scheme III. Thus, 2,6-dichlorobenzoic acid (Formula F; available from Aldrich Chemical Co.) is reacted with chlorosulfonic acid to yield the intermediate sulfonyl chloride (Formula G). The electrophilic sulfonyl chloride is reacted with a variety of amines to give the substituted benzoic acids (Formula 3).

Intermediate compounds for use in making the final compounds of the present invention are described in Examples 1 - 37.

Example 1N-Benzoyloxycarbonyl-L-aspartic acid bromomethyl ketone β -tert-butyl ester (Formula B)

5 To a solution of N-benzoyloxycarbonyl L-aspartate β -tert-butyl ester (Formula A; 10 g, 31 mmol) in 70 ml of anhydrous THF at -15 °C was added N-methyl morpholine (4.7 ml, 43.4 mmol) followed by the dropwise addition of ethyl chloroformate (3.9 ml, 40.5 mmol). The reaction mixture was stirred for 30 min at -15 °C and the suspension treated with diazomethane in ether (160 ml of a 0.4 in solution in ether, prepared from "DIAZALD®" [Aldrich]) and warmed to room temperature.

10 The bromomethyl ketone was formed in the same pot by cooling the intermediate diazoketone above followed by the dropwise addition of a 1:1 solution of 48% hydrobromic acid and glacial acetic acid (62 ml). After stirring for 15 min the reaction mixture was poured into a separatory funnel. The aqueous layer was drawn off and discarded. The remaining organic phase was washed with water, saturated aqueous NaHCO₃, brine and dried (MgSO₄). The solvents were removed in vacuo and the title compound so obtained (m.p. 41
15 -43 °C) was used in the subsequent displacement reactions without further purification.

Example 2N-Benzoyloxycarbonyl-L-Aspartic Acid 2,6-dichlorobenzoyloxymethylketone β -tert-Butyl Ester (Formula C)

20 The product of Example 1 (0.30 g; 0.76 mM) was dissolved in 12 ml of anhydrous DMF. To this solution was added powdered potassium fluoride (0.11 g; 19 mmol) and 2,6-dichlorobenzoic acid (0.17 g; 0.91 mmol) and the reaction mixture was stirred overnight. The solution was diluted with Et₂O and washed with water, aqueous saturated NaHCO₃, brine and dried (MgSO₄). The ketone so obtained was purified by silica
25 gel chromatography using ethyl acetate/hexane as the eluting solvent (¹H NMR (CDCl₃) δ 7.35 (m, 8H)), 5.90 (d, 2H each), 5.20 (m, 4H), 4.70 (m, 1H), 3.00 and 2.75 (doublet of doublets, 1H each), 1.42 (m, 9H).

In a similar manner, the following compounds of formula C were prepared:

Example 3

30 N-Benzoyloxycarbonyl-L-aspartic acid 2,6-difluorophenoxymethyl ketone β -tert-butyl ester from the product of Example 1 and 2,6-difluorophenol (mp 50-51 °C).

Example 4

35 N-Benzoyloxycarbonyl-L-aspartic acid 2,6-ditrifluoromethyl benzyloxymethyl ketone β -tert-butyl ester from the product of Example 1 and 2,6-ditrifluoromethyl benzoic acid (mp 62-63 °C).

Example 5

40 N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dichlorophenoxymethyl ketone β -tert-butyl ester from the product of Example 1 and 2,6-dichlorophenol.

Example 6

45 N-Benzoyloxycarbonyl-L-aspartic acid 2-fluoro-4-(N-morpholinyl sulfonamido)phenoxymethyl ketone β -tert-butyl ester from the product of Example 1 and 2-fluoro-4-(N-morpholinylsulfonamido)phenol.

Example 7

50 N-Benzoyloxycarbonyl-L-aspartic acid 2-chloro-4-(N-thio-morpholinylsulfonamido)phenoxymethyl ketone β -tert-butyl ester from the product of Example 1 and 2-chloro-4-(N-thio-morpholinylsulfonamido)phenol.

Example 8

55 N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dichloro-3-(2-N-morpholinylethoxy)benzoyloxymethyl ketone β -tert-butyl ester from the product of Example 1 and 2,6-dichloro-3-(2-N-morpholinylethoxy) benzoic acid.

Example 9

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dimethoxy benzoyloxymethyl ketone β -*tert*-butyl ester from the product of Example 1 and 2,6-dimethoxybenzoic acid.

Example 10

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dichloro-3-(benzyloxy) benzoyloxymethyl ketone β -*tert*-butyl ester from the product of Example 1 and 2,6-dichloro-3-(benzyloxy)benzoic acid.

Example 11

N-Benzoyloxycarbonyl-L-aspartic acid 2-acetamido-6-chlorobenzoyloxymethyl ketone β -*tert*-butyl ester from the product of Example 1 and 2-acetamido-6-chlorobenzoic acid.

Example 12

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-difluorobenzoyloxymethyl ketone β -*tert*-butyl ester from the product of Example 1 and 2,6-difluorobenzoic acid.

Example 13

N-Benzoyloxycarbonyl-L-aspartic acid 3-(N-butyl-sulfonamido)-2,6-dichlorobenzoyloxymethyl ketone β -*tert*-butyl ester from the product of Example 1 and 3-(N-butylsulfonamido)-2,6-dichlorobenzoic acid.

Example 14

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dichloro-3-sulfonamido benzoyloxymethyl ketone β -*tert*-butyl ester from the product of Example 1 and 2,6-dichloro-3-sulfonamidobenzoic acid.

Example 15

N-Benzoyloxycarbonyl-L-aspartic acid- 3-(N-benzyl-sulfonamido)-2,6-dichlorobenzoyloxymethyl ketone β -*tert*-butyl ester from the product of Example 1 and 2,6-dichloro-3-(N-benzylsulfonamido)benzoic acid.

Example 16

N-Benzoyloxycarbonyl-L-aspartic acid 3-(N-[2-aminoacetamidoyl] sulfonamido)-2,6-dichlorobenzoyloxymethyl ketone β -*tert*-butyl ester from the product of Example 1 and 3-(N-[2-aminoacetamidoyl] sulfonamido)-2,6-dichlorobenzoic acid.

Example 17

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dichloro-3-(N-morpholinylsulfonamido) benzoyloxymethyl ketone β -*tert*-butyl ester from the product of Example 1 and 2,6-dichloro-3-N-morpholinyl sulfonamido)benzoic acid.

Example 18

N-Methoxycarbonyl-L-Alanine-L-Aspartic Acid 2,6-dichlorobenzoyloxymethyl Ketone β -*tert*-Butyl Ester - (Formula E)

Part A: N-benzoyloxycarbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -*tert*-butyl ester (product of Example 2) (1.02 g, 2 mmol) was dissolved in absolute ethanol (100 ml) containing 6 N aqueous HCl (0.67 ml, 4 mmol). 10% Palladium on carbon (96 mg) was added and the reaction mixture was stirred under an ambient atmosphere of hydrogen gas for approximately 1 hour (thin layer chromatography [5% MeOH-CH₂Cl₂] indicated the disappearance of starting material). The solution was filtered and the solvent was removed *in vacuo* to give L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -*tert* butyl ester-HCl salt (Formula D) which was used immediately in the subsequent reaction described in Part B.

Part B: A solution of N-methoxycarbonyl-L-alanine (301 mg, 2.05 mmol) in CH₂Cl₂ (10 ml) was cooled to -20 °C and isobutylchloroformate (0.28 ml, 2.05 mmol) and N-methylmorpholine (0.23 ml, 2.05 mmol) were added sequentially. The reaction mixture was stirred for 15 minutes and a solution of the product of Part A above added followed by a second addition of N-methyl morpholine (0.23 ml, 2.05 mmol).

The reaction mixture was stirred for 30 minutes and was then diluted with EtOAc, washed with water, aqueous saturated NaHCO₃, brine and dried (MgSO₄). The solvents were removed *in vacuo* and the product purified by silica gel chromatography using 40% EtOAc/hexane as eluent to give the desired end-product (0.72 g; 80%).

In a similar fashion the following compounds of Formula E were prepared:

Example 19

N-(2-Thienyl)carbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -tert-butyl ester from the product of Example 2 and 2-thiophene carboxylic acid.

Example 20

N-Methoxycarbonyl glycine-L-aspartic acid 2,6-dichlorobenzoyloxymethylketone β -tert-butyl ester from the product of Example 2 and N-methoxycarbonyl glycine.

Example 21

N-Methoxycarbonyl-L-phenylalanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -tert-butyl ester from the product of Example 2 and N-methoxycarbonyl-L-phenyl alanine.

Example 22

N-Methoxycarbonyl L- β -(2-thienyl)alanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -tert-butyl ester from N-benzyloxycarbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -tert-butyl ester and N-methoxycarbonyl-L- β -(2-thienyl)alanine.

Example 23

N-Methoxycarbonyl-L-valine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -tert-butyl ester from the product of Example 2 and N-methoxycarbonyl-L-valine.

Example 24

N-Methoxycarbonyl-L-histidine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -tert-butyl ester from the product of Example 2 and N-methoxycarbonyl-L-histidine.

Example 25

N-Benzyloxycarbonyl-L-valine-L-aspartic acid 2,6-dichlorobenzoyloxymethylketone β -tert-butyl ester from the product of Example 2 and N-benzyloxycarbonyl-L-valine.

Example 26

N-Benzyloxycarbonyl-L-alanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -tert-butyl ester from the product of Example 2 and N-benzyloxycarbonyl-L-alanine.

Example 27

Benzyloxycarbonyl-L-valine-L-alanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -tert-butyl ester from the product of Example 2 and N-benzyloxycarbonyl-L-valine-L-alanine.

Example 28

N-2-Furoyl-L-Aspartic Acid 2,6-Dichlorobenzoyloxymethyl-ketone β -tert-Butyl Ester (Formula E)

Part A: L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -tert-ester-HCl salt (Formula D) was prepared as described in Example 18 Part A and was used immediately in the subsequent reaction described in Part B.

Part B: To a solution of the product of Part A above (2.0 mmol) in CH_2Cl_2 (10 ml) at 0°C was added 2-furoyl chloride (0.21 ml, 2.05 mmol). N-methylmorpholine (0.25 ml; 2.10 mmol) was then added and the reaction mixture stirred for 1 hour as it slowly was allowed to warm to room temperature. The solution was diluted with EtOAc, washed with water, saturated aqueous NaHCO_3 , brine and dried (MgSO_4). The solvents were removed *in vacuo*. The product was purified by silica gel chromatography using 30% EtOAc-hexane as eluent to give the desired end-product (mp $73-74^\circ\text{C}$).

In a similar fashion the following compounds of Formula E were prepared:

15 **Example 29**

N-2-Furonylcarbonyl-L-aspartic acid 2,6-dichloro-3-(N-morpholiny)sulfonamido benzoyloxymethyl ketone β -tert-butyl ester from the product of Example 17 and 2-furoic acid chloride.

20 **Example 30**

N-(3-Phenylpropionyl)-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -tert-butyl ester from the product of Example 2 and 3-phenylpropionyl chloride.

25 **Example 31**

N-Methoxycarbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -tert-butyl ester from the product of Example 2 and methyl chloroformate.

30 **Example 32**

N-(N,N-4-Dimethylaminomethyl)benzoyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β -tert-butyl ester (m.p. $63-65^\circ\text{C}$) from the product of Example 2 and 4-(N,N-dimethylaminomethyl)benzoyl chloride.

35 **Example 33**

3-(N-Butylsulfonamidoyl)-2,6-dichlorobenzoic acid (Formula 3)

Part A: Under an atmosphere of nitrogen gas, a reaction vessel was charged with 2,6-dichlorobenzoic acid (10 g, 53.55 mmol) (Formula F) and chlorosulfonic acid (3 ml, 472 mmol). The reaction mixture was refluxed for 1 hour and cooled to 10°C . The contents of the reaction vessel were poured slowly into 3 l of ice water. The white solid which precipitated was collected by filtration and dried *in vacuo* (10 mm) at 35°C for 48 hours to give 3-(chlorosulfonyl)-2,6-dichlorobenzoic acid (Formula G) (9.2 g, 61% yield).

Part B: The product from Part A (1.5 g; 5.2 mmol) was dissolved in anhydrous toluene (35 ml) to which was added powdered K_2CO_3 (1.44 g; 10.4 mmol) and n-butylamine (1.0 ml, 10.4 mmol). The reaction mixture was stirred at 25°C for 12 hours. The solution was diluted slowly with 1 M ethereal HCl (20ml) and was then stirred for 30 minutes. The solution was filtered and the resulting filtrate was evaporated to dryness to give crude product. Further purification of the material by silica gel chromatography using EtOAc as the eluent provided the desired end-product. (1.43 g, 85%. ^1H NMR (DMSO) δ 8.11 (t, 1H), 7.98 and 7.71 (doublets, 1H each), 2.75 (m, 2H), 1.55 (m, 2H), 1.32 (m 2H), 0.87 (t, 3H).

In a similar manner, the following compounds of Formula 3 were prepared:

Example 34

55 2,6-Dichloro-3-sulfonamidoylbenzoic acid (^1H NMR (DMSO) δ 8.11 (t, 1H), 7.42 and 7.15 (doublets, 1H each), 7.26 (d, 2H) from the product of Example 33 Part A and 40% aqueous ammonium hydroxide.

Example 35

3-(N-Benzylsulfonamidoyl)-2,6-dichlorobenzoic acid (^1H NMR (DMSO) δ 8.70 (t, 1H), 7.90 and 7.65 (doublets, 1H each), 7.25 (m, 5H), 4.15 (m, 2H) from the product of Example 33 Part A and benzylamine.

Example 36

3-(N-[2-Aminoacetamido]sulfonamidoyl)-2,6-dichlorobenzoic acid from the product of Example 33 Part A and glycnamide (m. p. 210-213 °C).

Example 37

3-(N-Morpholino)sulfonamidoyl)-2,6-dichlorobenzoic acid from the product of Example 33 Part A and morpholine.

Example 38

N-Benzoyloxycarbonyl-L-Aspartic Acid 2,6-Dichlorobenzoyloxymethyl Ketone (Formula I)

A solution of β -*tert*-butyl ester of N-benzoyloxycarbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (Example 2) in methylene chloride containing 25% v/v trifluoroacetic acid (20 ml) was stirred for 2 hours at 0 °C. The solvent was removed in vacuo and the residue azeotroped three times with methylene chloride to give analytically pure end-product (high resolution mass spectrum for $\text{C}_{20}\text{H}_{17}\text{Cl}_2\text{NO}_7$ found 453.1572).

In a similar fashion, the following compounds of Formulae 1 and 2 were prepared:

Example 39

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-difluorophenoxymethyl ketone (high resolution mass spectrum for $\text{C}_{19}\text{H}_{17}\text{F}_2\text{NO}_6$ found 393.3562) from the β -*tert*-butyl ester of Example 3.

Example 40

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-difluoromethyl benzoyloxymethyl ketone (high resolution mass spectrum for $\text{C}_{22}\text{H}_{17}\text{O}_7\text{F}_6$ found 521.1452) from the β -*tert*-butyl ester of Example 4.

Example 41

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dichlorophenoxymethylketone (mass spectrum m/z 426 (M + H) from the β -*tert*-butyl ester of Example 5.

Example 42

N-Benzoyloxycarbonyl-L-aspartic acid 2-fluoro-4-(N-morpholinyl sulfonamido)phenoxymethyl ketone (m.p. 65-66 °C) from the β -*tert*-butyl ester of Example 6.

Example 43

N-Benzoyloxycarbonyl-L-aspartic acid 2-chloro-4-(N-thiomorpholinylsulfonamido)phenoxymethyl ketone - (m.p. 180-181 °C) from the β -*tert*-butyl ester of Example 7.

Example 44

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dichloro-3-(2-N-morpholinylethoxy)benzoyloxymethyl ketone - (high resolution mass spectrum for $\text{C}_{26}\text{H}_{23}\text{O}_9\text{N}_2\text{Cl}_2$ found 583.1245) from the β -*tert*-butyl ester of Example 8.

Example 45

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dimethoxybenzoyloxy methyl ketone (high resolution mass spectrum for $C_{22}H_{24}O_5N$ found 446.1430) from the β -*tert*-butyl ester of Example 9.

Example 46

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dichloro-3-(benzyloxy) benzoyloxymethyl ketone (high resolution mass spectrum for $C_{27}H_{24}O_8NCl_2$ found 560.0865) from the β -*tert*-butyl ester of Example 10.

Example 47

N-Benzoyloxycarbonyl-L-aspartic acid 2-acetamido-6-chlorobenzoyloxymethyl ketone (high resolution mass spectrum for $C_{22}H_{22}O_8N_2Cl_2$ found 477.1044) from the β -*tert*-butyl ester of Example 11.

Example 48

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-difluorobenzoyloxymethyl ketone (high resolution mass spectrum for $C_{20}H_{18}O_7NF_2$ found 422.1046) from the β -*tert*-butyl ester of Example 12.

Example 49

N-Benzoyloxycarbonyl-L-aspartic acid 3-(N-butyl-sulfonamido)-2,6-dichlorobenzoyloxymethyl ketone - (m.p. 48-50 °C) from the β -*tert*-butyl ester of Example 13.

Example 50

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dichloro-3-sulfonamido benzoyloxymethyl ketone (m.p. 44-46 °C) from the β -*tert*-butyl ester of Example 14.

Example 51

N-Benzoyloxycarbonyl-L-aspartic acid 3-(N-benzyl-sulfonamido)-2,6-dichlorobenzoyloxymethyl ketone - (m.p. 66-68 °C) from the β -*tert*-butyl ester of Example 15.

Example 52

N-Benzoyloxycarbonyl-L-aspartic acid 3-(N-[2-aminoacetamidoyl] sulfonamido)-2,6-dichlorobenzoyloxymethyl ketone (m.p. 54-56 °C) from the β -*tert*-butyl ester of Example 16.

Example 53

N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dichloro-3-(N-morpholinylsulfonamido)benzoyloxymethyl ketone (high resolution mass spectrum for $C_{24}H_{25}O_{10}N_2Cl_2$ found 603.0594) from the β -*tert*-butyl ester of Example 17.

Example 54

N-Methoxycarbonyl-L-alanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (Anal. calc. for $C_{17}H_{18}O_8Cl_2N_2$: C, 45.45; H, 4.04; N, 6.24. Found: C, 45.20; H, 4.06; N, 5.98) from the β -*tert*-butyl ester of Example 18.

Example 55

N-(2-thienyl)carbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (mass spectrum m/z 430 ($M+$)) from the β -*tert*-butyl ester of Example 19.

Example 56

5 N-Methoxycarbonyl glycine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (Anal. calc. for $C_{16}H_{16}O_8Cl_2N_2$: C, 44.16; H, 3.17; N, 6.44. Found: C, 44.24; H, 3.15; N, 6.12) from the β -*tert*-butyl ester of Example 20.

Example 57

10 N-Methoxycarbonyl-L-phenylalanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (Anal. calc. for $C_{23}H_{22}O_8Cl_2N_2$: C, 52.59; H, 4.22; N, 5.33. Found: C, 52.98; H, 4.38; N, 5.21) from the β -*tert*-butyl ester of Example 21.

Example 58

15 N-Methoxycarbonyl-L- β -(2-thienyl)alanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (mass spectrum m/z 531 ($M+$)) from the β -*tert*-butyl ester of Example 22.

Example 59

20 N-Methoxycarbonyl-L-valine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (m.p. 119-120°C) from the β -*tert*-butyl ester of Example 23.

Example 60

25 N-Methoxycarbonyl-L-histidine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (Anal. calc. for $C_{22}H_{21}O_{10}F_3Cl_2N_4$: C, 41.99; H, 3.36; N, 8.90. Found: C, 42.08; H, 3.48; N, 8.67; mass spectrum m/z 515 ($M+$)) from the β -*tert*-butyl ester of Example 24.

Example 61

30 N-Benzoyloxycarbonyl-L-valine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (Anal. calc. for $C_{25}H_{26}O_8Cl_2N_2$: C, 54.26; H, 4.47; N, 5.06. Found: C, 54.06; H, 4.74; N, 4.91) from the β -*tert*-butyl ester of Example 25.

Example 62

35 N-Benzoyloxycarbonyl-L-alanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (mass spectrum m/z 525 ($M+$)) from the β -*tert*-butyl ester of Example 26.

Example 63

40 N-Benzoyloxycarbonyl-L-valine-L-alanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (Anal. calc. for $C_{28}H_{31}O_9Cl_2N_3$: C, 53.85; H, 5.00; N, 6.73. Found: C, 54.00; H, 5.04; N, 6.66) from the β -*tert*-butyl ester of Example 27.

Example 64

45 N-(2-Furonyl)carbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (mass spectrum m/z 414 ($M+$)) from the β -*tert*-butyl ester of Example 28.

Example 65

50 N-(2-Furonyl)carbonyl-L-aspartic acid 2,6-dichloro-3-(N-morpholinylsulfonamido)benzoyloxymethyl ketone (mass spectrum m/z 563 ($M+$)) from the β -*tert*-butyl ester of Example 29.

55

Example 66

N-(3-Phenylpropionyl)-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (¹H NMR (CDCl₃) δ 7.40 (m, 9H), 5.05 (2xdd, 4H), 4.70 (m, 1H), 2.85 (m, 2H), 2.65 (dd, 1H), 2.60 (dd, 1H), 2.50 (m, 2H) from the β-*tert*-butyl ester of Example 30.

Example 67

N-Methoxycarbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (¹H NMR (DMSO) δ 7.60 (m, 6H), 5.24 (m, 4H), 4.51 (m, 1H), 3.58 (s, 3H), 2.75 (dd, 1H), 2.55 (dd, 1H) from the β-*tert*-butyl ester of Example 31.

Example 68

N-(4-N,N-dimethylaminomethyl)benzoyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (m.p. 55-57 °C) from the β-*tert*-butyl ester of Example 32.

Example 69

N-Benzyloxycarbonyl-D-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (high resolution mass spectrum for C₂₀H₁₇C₁₂NO₇, found 453.1583) from N-benzyloxycarbonyl-D-aspartic acid bromomethyl ketone β-*tert*-butyl ester and 2,6-dichlorobenzoic acid using the procedures described in Examples 1, 2 and 38.

Example 70

N-(2-[2,6-dichlorobenzoyloxy])acetyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone (mass spectrum m/z 551 (M⁺) from N-Benzyloxycarbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone β-*tert*-butyl ester and 2-(2,6-dichlorobenzoyloxy)acetic acid using the procedures described in Examples 18 and 38.

Example 71

N-Benzyloxycarbonyl-L-valine-L-aspartic acid 4-(N,N-diethylsulfonamido)-2,3,5,6-tetrafluorophenoxymethyl ketone (mass spectrum m/z 664 (M+H) from N-benzyloxycarbonyl-L-aspartic acid bromomethyl ketone β-*tert*-butyl ester (Formula B), N-benzyloxycarbonyl-L-valine and 4-(N,N-diethyl-sulfonamido)-2,3,5,6-tetrafluorophenol using the procedures described in Examples 2, 18 and 38. The 4-(N,N-diethyl-sulfonamido)-2,3,5,6-tetrafluorophenol was prepared by reacting 2,3,5,6-tetrafluorophenol with chlorosulfonic acid followed by reaction with diethylamine, analogous to the procedure described in Scheme III and Example 33.

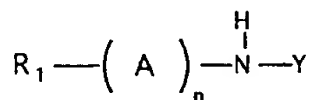
Compounds of the present invention were tested for IL-1β protease inhibition activity according to the following protocol:

Partially purified IL-1β protease is stored at -80 °C, thawed on ice, and preincubated for 10 minutes at 37 °C with 2.5 mM dithiothreitol in a buffer solution containing 10 mM Tris-HCl (pH 8.0) and 25% (v/v) glycerol. Inhibitors are prepared as stock solutions in dimethyl sulfoxide (DMSO). The protease is preincubated with inhibitor in a volume of 20 μl in a 1.5 ml polypropylene microcentrifuge tube for 15 minutes at 37 °C. The volume of compound added to the assay is adjusted to yield a DMSO concentration in the preincubation of <15% (v/v). The enzyme assay is then initiated by the addition of substrate (TRITC-AVVDAPVRS-NH₂) to yield a final concentration of 67 μM in a final volume of 30 μl. The reactions are carried out for 60 minutes at 37 °C in the dark and are terminated by the addition of 10 μl of 10% trifluoroacetic acid (TFA). Following the addition of 115 μl of 0.1% TFA, the samples are analyzed by high pressure liquid chromatography using a reverse phase (C18) column and elution with an acetonitrile/water/TFA gradient. Substrate and product are monitored by their absorbance at 550 nm and elute at 4.2 and 5.2 minutes, respectively.

Example No.	TABLE I IC ₅₀ μm	Name of Compound
38	N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone	0.05
40	N-Benzoyloxycarbonyl-L-aspartic acid 2,6-ditrifluoromethylbenzoyloxymethyl ketone	0.10
41	N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dichlorophenoxymethyl ketone	0.10
42	N-Benzoyloxycarbonyl-L-aspartic acid 2-fluoro-4-(N-morpholinylsulfonamido)phenoxymethyl ketone	0.32
49	N-Benzoyloxycarbonyl-L-aspartic acid 3-(N-butylsulfonamido)-2,6-dichlorobenzoyloxymethylketone	0.09
52	N-Benzoyloxycarbonyl-L-aspartic acid 3-(N-[2-aminoacetamidoyl]sulfonamido)-2,6-dichlorobenzoyloxymethyl ketone	0.06
53	N-Benzoyloxycarbonyl-L-aspartic acid 2,6-dichloro-3-(N-morpholinylsulfonamido)benzoyloxymethyl ketone	0.09
54	N-Methoxycarbonyl-L-alanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone	0.06
57	N-Methoxycarbonyl-L-phenylalanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone	0.07
64	N-(2-furonyl)carbonyl-L-aspartic acid 2, 6-dichlorobenzoyloxymethyl ketone	0.14
67	N-Methoxycarbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone	0.08
68	N-(4-(N,N-dimethylaminomethyl)benzoyl-L-aspartic acid 2, 6-dichlorobenzoyloxymethyl ketone	0.3
70	N-(2-[2,6-dichlorobenzoyloxy])acetyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone	0.2

Claims

1. A compound of the formula (I)

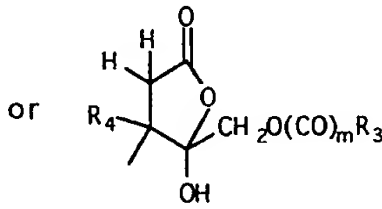
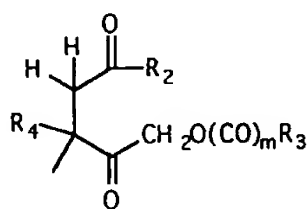


Formula I

or a pharmaceutically acceptable salt thereof, wherein

n = 0-4;

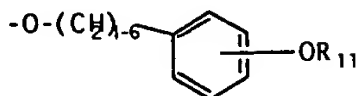
Y =



m = 0,1;

R₃ = a singularly or multiply substituted aryl wherein aryl is a phenyl or naphthyl ring wherein the substituents are independently selected from the group consisting of:

- (1) H; (2) halogen; (3) OH
 (4) CF₃; (5) NO₂; (6) OR₅
 (7) COR₉; (8) NR₆COR₁₀; (9) CONR₆R₆
 (10) SO₂NR₆R₆; (11) SO₂R₆; (12) COOR₁₁;
 (13)



and
 (14) lower alkyl and lower cycloalkyl;

R₅ =

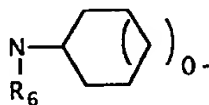
- (1) lower straight chain or branched alkyl, lower cycloalkyl;
 (2) (CR₆R₇)₀₋₆-aryl; (3) (CR₆R₇)₀₋₆-heteroaryl or (4) (CR₆R₇)₂₋₆-R₈;

R₆ and R₇

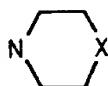
are independently H, lower straight chain or branched alkyl, benzyl, cycloalkyl or aryl wherein aryl is defined as above and heteroaryl includes pyridyl, thienyl, fury, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, benzimidazolyl, pyrazinyl, pyrimidyl, quinolyl, isoquinolyl, isothiazolyl, benzofuranyl, isoxazolyl, triazinyl and tetrazolyl;

R₈ =

- (1) OCH₂CH₂OR₆; (2) OCH₂CH₂NR₆R₇
 (3) NR₆CH₂CO₂R₆
 (4)



(5)



X = O, S and

(6) NR₆R₇

wherein R₆ and R₇ are as above defined;

R₉ =

- (1) lower straight chain or branched alkyl, lower cycloalkyl
 (2) (CR₆R₇)₀₋₆-aryl; (3) (CR₆R₇)₀₋₆-heteroaryl; or
 (4) (CR₆R₇)₀₋₆-R₈,
 wherein R₆, R₇ and R₈ are as above defined;

R₁₀ =

- (1) R₉; (2) OR₁₁; or (3) NR₆R₁₁,

wherein

R₁₁ =

- (1) lower straight chain or branched alkyl, lower cycloalkyl
 (2) (CR₆R₇)₁₋₆-aryl; (3) (CR₆R₇)₁₋₆-heteroaryl; or
 (4) (CR₆R₇)₂₋₆-R₈,
 wherein R₆, R₇, R₈, and R₉ are as above defined;

R₄ =

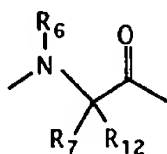
H or deuterium;

R₂ =

(1) OR_6 ; (2) NR_6OR_7 or
(3) NR_6R_7
wherein R_6 and R_7 are as above-defined;

A =

(1) an amino acid of the formula (II)



Formula 2

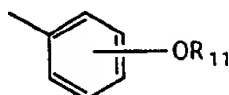
wherein R_6 and R_7 are as defined above;
independently

R_{12} is

(1) H; or (2) $(CR_6R_7)_{1-6}-R_{13}$,
wherein R_6 and R_7 are as above-defined;

R_{13} =

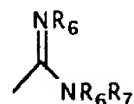
(1) H; (2) F; (3) CF_3
(4) OH; (5) OR_{11} ; (6) NR_6R_{14}
(7) cycloalkyl; (8) aryl
(9) heteroaryl; (10) SH
(11) SR_{11} ; (12) $CONR_5R_6$
(13) $COOR_5$ or
(14)



wherein R_5 , R_6 , R_{11} and R_{14} are as defined above;

R_{14} =

(1) R_7 ; (2) COR_{10} ; (3) $SO_2NR_5R_6$ or
(4)

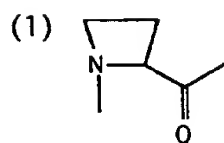


wherein R_5 , R_6 , R_7 and R_{10} are as defined above; or

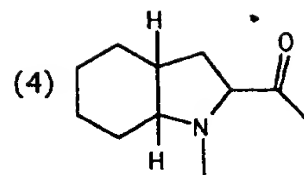
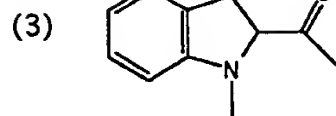
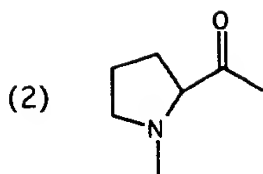
A =

(2) an amino acid selected from the group consisting of

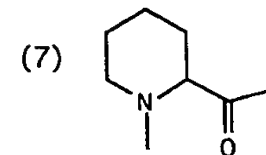
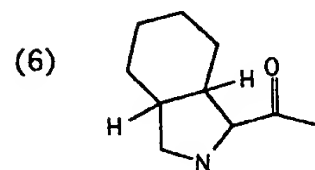
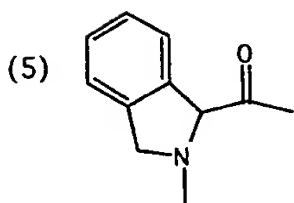
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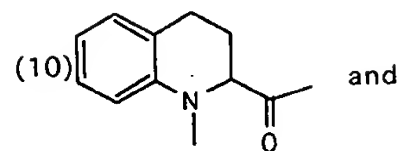
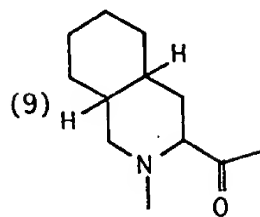
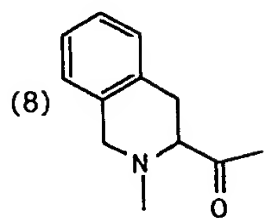


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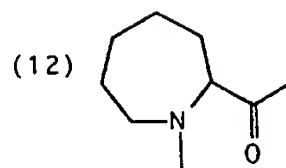
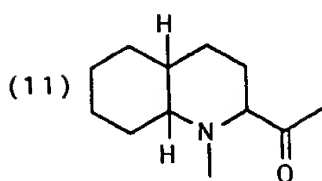
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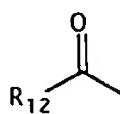
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R₁ is an acyl group of the formula (III)

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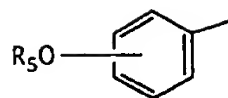
Formula III

wherein

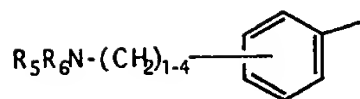
R₁₂ is

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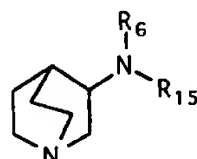
- (1) OR₅; (2) NR₅ R₆; (3) R₅; (4) -CH=CHR₅
(5)



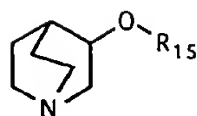
(6)



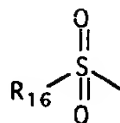
(7)



or

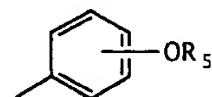


wherein
 R_{15} = single bond, $(CN_2)_{2-6}-NR_6-$, $(CN_2)_{2-6}-O-$ and R_5 and R_6 are as above defined; or a sulfonyl group of the formula (IV)

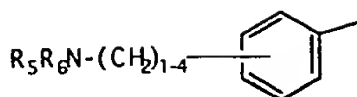


Formula IV

wherein
 R_{16} is
 (1) R_5
 (2)



or
 (3)



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wherein R_5 and R_6 are as above-defined.

2. A compound according to claim 1 selected from the group consisting of: N-Benzyloxycarbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 10 N-Benzyloxycarbonyl-L-aspartic acid 2,6-di fluorophenoxymethyl ketone,
 N-Benzyloxycarbonyl-L-aspartic acid 2,6-ditri fluoromethylbenzoyloxymethyl ketone,
 N-Benzyloxycarbonyl-L-aspartic acid 2,6-dichlorophenoxymethyl ketone,
 N-Benzyloxycarbonyl-L-aspartic acid 2-fluoro-4-(N-morpholinylsulfonamido)phenoxymethyl ketone,
 15 N-Benzyloxycarbonyl-L-aspartic acid 2-chloro-4-(N-thiomorpholinylsulfonamido)phenoxymethyl ketone,
 N-Benzyloxycarbonyl-L-aspartic acid 2,6-dichloro-3-(2-N-morpholinylethoxy) benzyloxymethyl ketone,
 N-Benzyloxycarbonyl-L-aspartic acid 2,6-dimethoxybenzoyloxy methyl ketone,
 N-Benzyloxycarbonyl-L-aspartic acid 2,6-dichloro-3-(benzyloxy)benzyloxymethyl ketone,
 N-Benzyloxycarbonyl-L-aspartic acid 2-acetamido-6-chlorobenzoyloxymethyl ketone,
 20 N-Benzyloxycarbonyl-L-aspartic acid 2,6-difluorobenzoyloxymethyl ketone,
 N-Benzyloxycarbonyl-L-aspartic acid 3-(N-butylsulfonamido)-2,6-dichlorobenzoyloxymethyl ketone,
 N-Benzyloxycarbonyl-L-aspartic acid 2,6-dichloro-3-sulfonamido benzoylmethyl ketone,
 N-Benzyloxycarbonyl-L-aspartic acid 3-(N-benzylsulfonamido)-2,6-dichlorobenzoyloxymethyl ketone,
 N-Benzyloxycarbonyl-L-aspartic acid 3-(N-[2-aminoacetamidoyl] sulfonamido)-2,6-dichlorobenzoylox-
 25 ymethyl ketone,
 N-Benzyloxycarbonyl-L-aspartic acid 2,6-dichloro-3-(N-morpholinylsulfonamido)benzoyloxymethyl ke-
 tone,
 N-Methoxycarbonyl-L-alanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 N-(2-thienyl) carbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 30 N-Methoxycarbonylglycine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 N-Methoxycarbonyl-L-phenylalanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 N-Methoxycarbonyl-L-β-(2-thienyl)alanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 N-Methoxycarbonyl-L-valine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 N-Methoxycarbonyl-L-histidine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 35 N-Benzyloxycarbonyl-L-valine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone.,
 N-Benzyloxycarbonyl-L-alanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 N-Benzyloxycarbonyl-L-valine-L-alanine-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 N-(2-furonyl)carbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 N-(2-furonyl)carbonyl-L-aspartic acid 2,6-dichloro-3-(N-morpholinylsulfonamido)benzoyloxymethyl ke-
 40 tone,
 N-(3-phenylpropionyl)-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 N-Methoxycarbonyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 N-(4-(N,N-dimethylaminomethyl)benzoyl)-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 N-benzyloxycarbonyl-D-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone,
 45 N-(2-[2,6-dichlorobenzoyloxy])acetyl-L-aspartic acid 2,6-dichlorobenzoyloxymethyl ketone, and
 N-Benzyloxycarbonyl-L-valine-L-aspartic acid 4-(N,N-diethyl-sulfonamido)-2,3,5,6-tetrafluorophenox-
 ymethyl ketone.
3. A pharmaceutical composition for inhibiting interleukin 1β protease comprising a compound of the
 50 formula (I) or a pharmaceutically acceptable salt thereof as claimed in any one of the preceding claims
 in combination with a pharmaceutically acceptable carrier.
4. A method of inhibiting interleukin-1β protease activity in a mammal in need of such treatment
 comprising administering to said mammal an effective inhibitory amount of a compound of formula (I)
 55 or a pharmaceutically acceptable salt thereof as claimed in any one of claims 1 to 7 or a pharmaceuti-
 cal composition thereof as claimed in claim 8.

5. The use of a compound of formula (I) or a pharmaceutically acceptable salt thereof as claimed in any one of claims 1 to 7 or a pharmaceutical composition thereof as claimed in claim 8 for the preparation of a medicament for inhibiting interleukin-1 β protease activity in a mammal.

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European Patent
Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention EP 94 20 1161
shall be considered, for the purposes of subsequent
proceedings, as the European search report

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
Y	NATURE vol. 356 , 30 April 1992 , LONDON GB pages 768 - 774 N. A. THORNBERRY ET AL. 'A novel heterodimeric cysteine protease is required for interleukin-1beta processing in monocytes' * page 768, right column, line 31 - line 43; page 769, left column, line 11; right column, line 1-2 * ---	1,2,8-10	C07C271/22 A61K31/27 C07D295/30 C07D295/088 C07C311/16 C07D333/38 C07D307/38 C07K5/06 C07K5/08 A61K31/18 A61K37/02
A	WO-A-91 15577 (IMMUNEX CORP.) * page 4, line 26 - page 5, line 10; claims 14,21 * --- -/--	1,2,8-10	
			TECHNICAL FIELDS SEARCHED (Int.Cl.5)
			C07C C07D
INCOMPLETE SEARCH			
<p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims</p> <p>Claims searched completely : Claims searched incompletely : Claims not searched : Reason for the limitation of the search:</p> <p>see sheet C</p>			
Place of search THE HAGUE		Date of completion of the search 27 July 1994	Examiner Seufert, G
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone V : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application I : document cited for other reasons A : member of the same patent family, corresponding document	



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Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 94 20 1161

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
Y,D	BIOCHEMISTRY vol. 30, no. 19 , 1991 , EASTON, PA US pages 4678 - 4687 A. KRANTZ ET AL. 'Peptidyl (acyloxy)methyl ketones and the quiescent affinity label concept: the departing group as a variable structural element in the design of inactivators of cysteine proteinases' * page 4679, compound IV, compounds in table I and II; page 4684, scheme II * ----	1,2,8-10	
A	EP-A-0 272 671 (SYNTEX) * abstract; claims * ----	1,8-10	
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 265, no. 24 , 1990 , BALTIMORE, MD US pages 14526 - 14528 P. R. SLEATH ET AL. 'Substrate specificity of the protease that processes human interleukin-1beta' ----	1,8-10	TECHNICAL FIELDS SEARCHED (Int.Cl.5)
P,X	BIOCHEMISTRY vol. 33, no. 13 , 5 April 1994 , EASTON, PA US pages 3934 - 3940 N. A. THONBERRY ET AL. 'Inactivation of interleukin-1beta converting enzyme by peptide (acyloxy)methyl ketones' * compounds table 1; compounds b, table 2 * ----- -/--	1	



EP 94 20 1161

-C-

Remark: Although claim 9
is directed to a method of
treatment of the human/animal
body (Art. 52(4) EPC) the search
has been carried out and based on
the alleged effects of the
compound/composition

